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RESEARCH PAPER

An assessment of response of soil-based indicators to nitrogen fertilizer across four tropical eucalyptus plantations

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Abstract: Low nitrogen (N) availability often results in reduced productivity of *Eucalyptus* plantations. We studied the response of four eucalyptus plantations (two plantations of *E. tereticornis* on the coastal lowlands, and two plantations of *E. grandis* in the upland region of the Western Ghats, Kerala, India) to N addition and related this response to seasonal N mineralization as well as other indices of N availability, in order to examine the utility of soil based indicators of N mineralization for predicting the response of eucalyptus growth to added N fertilizer. Several biochemical indicators were examined for their capacity to predict response to N fertilizer, including total soil N, soil C:N ratio, and N released during anaerobic and aerobic incubation. Results show that nitrogen fertilizer addition increased productivity across the 4 sites from 7% to 70%, N released during an aerobic incubation had the highest correlation with fertilizer response across the 4 sites (R^2=0.92, p<0.01), and that Modelled seasonal soil N mineralisation was a poorer predictor of fertilizer response than N released during an aerobic incubation. Whilst some of these indicators are promising, they need wider validation and testing before they could be routinely applied.

Keywords: nitrogen mineralization; *Eucalyptus grandis*; *Eucalyptus tereticornis*; N fertilization; site management; productivity improvement

Introduction

Eucalypts are one of the most widely planted exotic species in India, and are grown in varied climatic and soil conditions with an estimated total area of 8 $\times 10^6$ ha (FAO 2001). In Kerala, the most southwestern state of India, most of the earlier eucalypt plantations were established on fertile forest soils. The productivity of these plantations has declined over successive rotations, at least in part due to the export of large quantities of nutrients from the sites (Sankaran et al. 2005). Productivity of eucalyptus plantations can be improved through addition of nitrogen fertilizer (Sankaran et al. 2006), as has been found in other studies elsewhere (e.g. O'Connell et al. 2004), but the response is often highly dependent on site factors. Thus characterization of responsiveness of eucalyptus plantations to added N fertilizer is required to synchronize the N supply and demand during the growth period.

Response of fast-growing trees to applied fertilizer N mainly depends on the rate of N mineralisation in the soil. Seasonal rates of N mineralisation can be predicted through model simulations (Van Veen et al. 1984; Gonçalves et al. 1994), which may be useful for predicting the availability of soil mineral N to growing plants. O'Connell and Rance (1999) reported that it was possible to predict seasonal soil N mineralisation under Australian conditions through an understanding of the basal rate of N mineralization (a soil specific parameter), and modifying this for soil moisture and temperature on a daily basis during the year.

Diagnostics for predicting the response of eucalyptus plantations to nitrogen fertilizer are currently not generally available, although there is a strong imperative to apply fertilizer in excess to fertile sites. Thus supplementary N fertilizer must match seasonal variations in internal N requirement during the plantation growing period. The aim of this study was to examine the hypothesis that modelling seasonal N mineralization may be a useful diagnostic tool for identifying sites that will be responsive to N fertilizer application. We tested this hypothesis across 4 sites, recognising that this framework is sufficient only to test the hypothesis, rather than derive a robust index of N fertilizer response.

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Materials and methods

Study area

The study was conducted in the state of Kerala in south west of peninsular India. The study area has a tropical warm humid cli-

mate and receives an average annual rainfall of 3000 mm during the southwest and northeast monsoons. The maximum rainfall is obtained from the south west monsoon during June–October. The northeast monsoon brings occasional rains from December to February. Mean air temperature is 27 °C (range for 20–42 °C) and relative humidity ranges between 64% during February – March and 93% during June–July.

Establishment of field experiments

Four experimental sites were established in Kayampoovam and Punnala with *Eucalyptus tereticornis* at low elevation on the coastal plain (<150 m) and Vattavada and Surianelli with *E. grandis* in the high ranges of the Western Ghats mountains. The experimental site at Kayampoovam was originally a degraded moist deciduous forest where *E. tereticornis* was planted in 1977 and the trees were first harvested in 1991. The site was characterised by moderate rainfall (less summer rain), low humidity, high evaporation and high wind velocity. The site at Punnala had originally a degraded moist deciduous forest before planting eucalypt in 1977 with high humidity, high evaporation and low wind velocity. At Vattavada, eucalypt plantations were established in 1958 on cleared natural semi-evergreen (shola) forest. The experiment was laid out in 1991 after 3 rotations of *E. grandis*. The site selected at Surianelli was a grassland before planting eucalypt in 1978. The trees were clear-felled in 1991 after three rotations. The site was replanted in 1991 and harvested in May 1998. The details of the sites are listed in Table 1. The overall experimental design and other details are described elsewhere (Sankaran et al. 2005), but the focus of this study was on nitrogen response experiments to establish relationships with modelled and measured soil nitrogen indices.

Table 1. Climate and site characteristics of study area

Site and plants	Rainfall ($mm \cdot a^{-1}$)	Altitude (m)	Original vegetation	Soil texture	Soil pH	Soil C (0-10 cm) ($mg \cdot g^{-1}$)	Soil N (0-10 cm) ($mg \cdot g^{-1}$)	C:N ratio
Kayampoovam (*E. tereticornis*)	2700	120	Moist deciduous forest	Light to medium clay	5.3	21.5	1.83	11.8
Punnala (*E. tereticornis*)	2000	150	Moist deciduous forest	Sandy loam to clay loam	5.1	43.6	2.89	15.1
Surianelli (*E. grandis*)	3000	1280	Grassland	Medium clay to sandy loam	4.8	40.9	2.49	16.4
Vattavada (*E. grandis*)	1800	1800	Semi-evergreen forest	Clay loam to medium clay	5.3	52.3	4.50	11.6

At each site, a nitrogen fertilizer response experiment was established in a randomised block with 4 replicates. Plots of 20 m×20 m in size were established, each plot with 100 seedlings in total and an inner measurement block of 36 trees (2 buffer rows within each plot were not measured). At Kayampoovam, the plot size was 18 m×18 m due to limited land area available. Five rates of N fertilizer (0, 18, 60, 187, and 375 $kg \cdot ha^{-1} \cdot a^{-1}$) were added (3 times each year) for the first 2 years with a basal dressing (non-limiting quantities) of P, K and trace element fertilisers.

Aerobic index of soil N mineralisation

Soil samples were collected from 4 replicate plots adjacent to the N response experiment. Nine paired soil samples were collected from each replicate using soil cores inserted to a soil depth of 20 cm when the soils were near field capacity. Intact soil cores were transported to the laboratory in cooled and insulated boxes. One core from each pair was extracted immediately (the 'initial' sample), and the other core was incubated aerobically in the laboratory at 25°C for 14 days and then extracted (the 'final' sample). At each time of extraction, soil was pushed out as intact core and separated into depths of 0–5 and 5–10 cm. Within each depth range, the nine cores per plot were aggregated and passed through a 5-mm sieve. Sub-samples were dried at 105 ºC for moisture content determination. To extract the mineral nitrogen, approximately 20 g of fresh soil was taken from each sample, and extracted with 60 mL of 1M KCl after shaking for 1 hr on an end-over-end shaker. Mineral ammonium and nitrate in the extracts were analyzed by automated colorimetry (Rayment et al. 1992). The net N mineralized during the 14-day incubation was determined as the difference in mineral N content between 'initial' and 'final' samples.

Anaerobically mineralizable N was determined by adding 30-mL distilled water to 20-g moist weight of the 'initial' soil and incubating for seven days at 40°C (Keeney et al. 1966). Ammonium was extracted by shaking for one hr after adding 30 mL of 2-M KCl. Anaerobically mineralizable N was calculated from the difference in ammonium content of pre and post incubated samples.

Response of N mineralization to soil moisture

The effect of soil moisture on soil N mineralization was evaluated in a laboratory study, using the soil samples collected as above. The soils were pushed out of the cores, dried, and repacked into mini-cores suitable for adjusting the moisture on a pressure plate. The mini-cores were placed on pressure plates and established at 10 different matric potentials (-10, -25, -50, -100, -400, -1500 Kpa, and 4 levels of air drying), with 2 replicates. Two cores of each combination were set up, one for immediate extraction, and another for extraction after incubation. In total, 160 cores were established. Soil cores were equilibrated on pressure plates at 4°C for 48 h to arrive at desired moisture contents. After equilibration, half of the cores were extracted immediately, and half were incubated at 25°C and for 14 days prior to extraction. For the extraction, 20 g of moist soil was extracted with 60 mL of 1-N KCl. The available N (NH_4-N and NO_3-N) content was determined and expressed on oven dry weight basis. The relationship between soil moisture and N mineralization was fitted to a sigmoidal function of the following form:

$$y = a + \frac{c}{1 + e^{-b(x-M)}} \quad (1)$$

where y is the N mineralization relative to that in non-water limited soil (unit-less scale of 0-1), x is the soil moisture content relative to that at -10 KPa matric potential (also unit-less), and a, c, and M are fitted coefficients.

Response of N mineralization to soil temperature

A separate laboratory incubation experiment was conducted to study the response of N mineralisation to a range of temperatures viz, 14, 20, 25, 30, and 35°C. Soil samples were prepared as above and then equilibrated at 4°C on a pressure plate at matric potential of -25 Kpa for 48 h. Two replicates of each site were incubated in each of the temperatures. In total, 40 cores were incubated, and another 16 (4 per site) were extracted immediately after equilibration for the baseline mineral N. The incubations were conducted over variable times to account for the different rates of N mineralization, with the cores incubated at 35°C for 7 days, the cores incubated at 25 and 30°C for 14 days, and the remainder were incubated for 28 days. At the end of incubation period, 20-g moist soil was extracted with 60 mL of 1N KCl and analyzed colourimetrically for NH_4-N and NO_3-N. Net N mineralized during the incubation was calculated as the mineral N at the end of the incubation after subtracting the initial mineral N pool. The relationship between soil temperature and N mineralization was fitted to a exponential function of the following form:

$$y = a + b \times r^{x} \quad (2)$$

where, y is the N mineralization relative to that at 25°C (unit-less), x is the soil temperature (°C), and a, b, and r are fitted coefficients.

Field temperature and moisture

Site environmental conditions were measured between July/August 1998 and February/March 2000 at each of the 4 sites. For this reason we focussed on the 12 months of calendar year 1999 for modelling. Soil cores were collected from the field at 28-day intervals to measure gravimetric moisture content and soil temperature was measured at depth of 5 cm at 9 a.m. and 3 p.m. on a weekly basis.

Seasonal N mineralization modelling

Soil N mineralisation (N_{min}) is predicted on a daily basis using the following equation:

$$N_{min} = N_{index} \times T \times M \quad (3)$$

where N_{index} is the aerobic index of N mineralization described above, T is a temperature modifier, and M is a soil moisture modifier. The temperature and moisture modifiers mimic the conditions as in the field situations, which is incorporated in the equations described above.

Tree growth

Trees were measured at regular intervals during the rotation, but for the purposes of this study we used the standing volume at 4 years to assess plantation response to N fertilizer. Standing volume was calculated as the sum of the tree volumes in each plot. Individual over-bark tree volume (V) was calculated according to the equation:

$$V = {}^{1}/_{3}\pi r^{2}h \quad (4)$$

where each tree was assumed to be conical in shape, with a base radius (r) and height (h).

Response to fertilizer (%) at each site was calculated based on the growth data in control and non-N-limited plots.

Statistical analysis

The Genstat statistical package was used for all analyses, including analysis of variance to assess the significance of treatments on tree growth, and regression analysis to test the significance of the productivity response to soil N indices.

Results and discussion

Indices of soil N mineralization

The aerobic and anaerobic indices of N availability showed different trends across the sites (Table 2). The aerobic N index was lowest at Surianelli and highest at Vattavada (and intermediate at the two sites of *E. tereticornis*), and Vattavada also had the highest anaerobic N index followed by Surianelli.

Table 2. Soil N indices across the 4 sites

Site	Aerobic N($\mu g \cdot g^{-1} \cdot a^{-1}$)	Anaerobic N ($\mu g \cdot g^{-1} \cdot a^{-1}$)
K'poovam (*E. tereticornis*)	277.4	13505.0
Punnala (*E. tereticornis*)	255.5	7774.3
Surianelli (*E. grandis)*	129.6	26097.5
Vattavada (*E. grandis)*	489.1	54859.5

Effects of soil moisture and temperature on net N mineralization

The relationships between N mineralization and soil moisture and temperature under laboratory condition with soils from across the 4 sites are shown in Fig. 2. A sigmoidal function was fitted to the moisture response across all sites (R^2 of 0.83, Fig. 1(a)). The shape of the curve suggests that N mineralization continued to increase with increasing soil water content above -25 kPa.

The temperature response experiment showed that N mineralization rate was relatively dropped below 25°C, doubled between 25°C and 35°C and continued to increase exponentially up to 35°C in all the four sites. The response varied significantly between the lowland (*E. tereticornis*) soils and the upland (*E. grandis*) soils; therefore separate non-linear parameters were

fitted (overall R^2 was 0.983, Fig. 1(b)). These moisture and temperature response functions were used with measured soil moisture and temperature data to model seasonal N mineralisation.

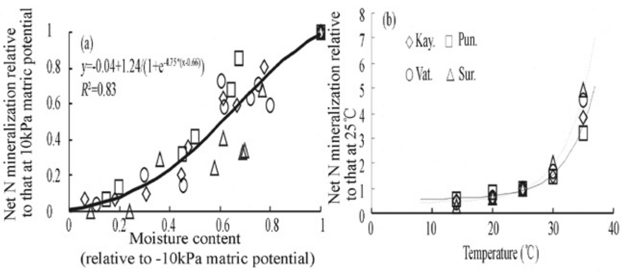

Fig. 1 Response of relative net N mineralization to (a) soil moisture content (relative to that at matric potential of -10 kPa), and (b) temperature in a laboratory incubation (relative to that at 25 °C)

Modelled net N mineralisation

The seasonal and rate of predicted net N mineralization varied across the sites for the calendar year 1999 (Fig. 2), with maximum N mineralization response at Kayampoovam (118 kg·ha^{-1}·a^{-1}) followed by Vattavada (112 kg·ha^{-1}·a^{-1}) and Punnala (107 kg·ha^{-1}·a^{-1}), and the lowest N mineralization was predicted at Surianelli for 26 kg·ha^{-1}·a^{-1}. The southwest monsoon (from June to September) had a large impact on N mineralization at Kayampoovam and Punnala, while at Vattavada, N mineralization was greater during the northeast monsoon (October to January) and both monsoons had a significant impact on predicted N mineralisation at Surianelli. There was distinct seasonal variation in N mineralization across the site, which was mostly due to the changes in soil moisture content over the year.

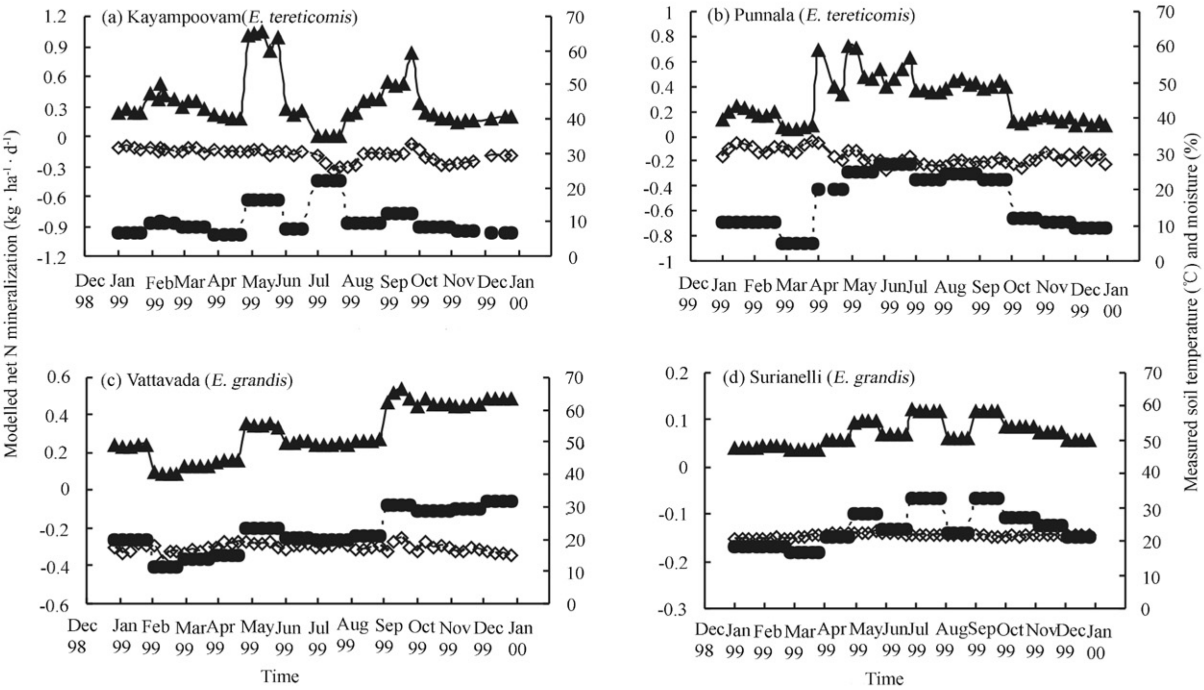

Fig. 2 Modelled N mineralization rates (0-10 cm) at (a) Kayampoovam, (b) Punnala, (c) Vattavada, and (d) Surianelli, in relation to the measured soil temperature and moisture during the course of the calendar year 1999.

Plant response to fertiliser N

Significant growth responses to added N were found at Kayampoovam, Punnala and Surianelli at 4 years (Table 3). Maximum productivity was obtained in the 60-kg·ha^{-1}·a^{-1} treatment at Kayampoovam, 187-kg·ha^{-1}·a^{-1} treatment at Punnala and Vattavada, and in the 375-kg·ha^{-1}·a^{-1} treatment at Surianelli (Fig. 3). The maximum response to N fertilizer was found at Surianelli (70%), whilst the response was between 42%–45% at Punnala and Kayampoovam. The basal productivity at Vattavada was high (MAI of 52 m^3·ha^{-1}·a^{-1} up to ages of 4 and those trees did not respond significantly to added N fertilizer.

Table 3. Growth at 4 years (m^3·ha^{-1}) with and without N fertilizer applied

Eucalyptus species/Site	Nil N	Sufficient N	Response to N fertilizer (%)	Significance level[a]
E. tereticornis				
Kayampoovam	64.6	93.8	45.2	**
Punnala	56.9	80.6	41.7	***
E. grandis				
Vattavada	210.5	226	7.4	ns
Surianelli	94.7	160.7	69.7	***

Notes: [a]Significance level denoted by: ** (<0.01), *** (<0.001), ns (not significant).

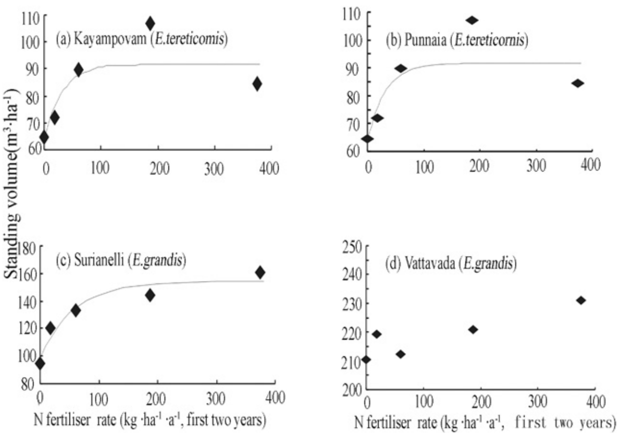

Fig. 3 Plantation productivity response at 4 years to N fertilizer application

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The range of responses to N fertilizer across the 4 sites (7%−70%) was useful for testing the potential generality of relationships between soil N indices and response of growth of eucalyptus to N fertilizer, although it is recognized that 4 sites are insufficient for developing a practical diagnostic tool. However, we have used this framework to screen a range of possible N indices for their potential application to a larger response dataset.

Relationship between response to fertiliser N and indices of soil N supply

Several measures of soil N availability were significantly related to fertilizer response across the 4 sites (Fig. 4). The C:N ratio (Fig. 4(a)) and total soil N (Fig. 4(b)) were broadly related to fertilizer response, although the *E. tereticornis* planted sites (Kayampoovam and Punnala) had similar fertilizer response to differing C:N ratios. The aerobic N mineralization index (Fig. 4(c)) was highly correlated with response to fertilizer (R^2 = 0.927), whilst anaerobically mineralisable N was not related to fertilizer response across the 4 sites (Fig. 4(d)). The Vattavada site had an N mineralization index of 1.34 $\mu g \cdot g^{-1} \cdot d^{-1}$, and a minimal response to N fertilizer, suggesting that this level of basal N mineralization was adequate to sustain maximum site potential. Of the 4 chemical/biological indices of N availability that were assessed, anaerobic N was the least well correlated with response to N fertilizer. The results corroborate the findings of Scott et al. (2005), that anaerobic N was not related to N fertilizer response in short-rotation *Liquidambar styraciflua* plantations across a range of soils in southern USA. Curtin and McCallum (2004) also found anaerobically mineralizable nitrogen to be of little value for predicting N supply in agricultural soils, although some studies have found anaerobic N to be more useful in other situations (eg. Stockdale and Rees 1994). Total N, soil C:N ratio and aerobic N mineralization showed more promise as indicators of response to N fertilizer, with the best indicator being the aerobic N mineralization index, which again supports the findings of both Scott et al. (2005) and Curtin and McCallum (2004) that it is highly correlated with soil N supply.

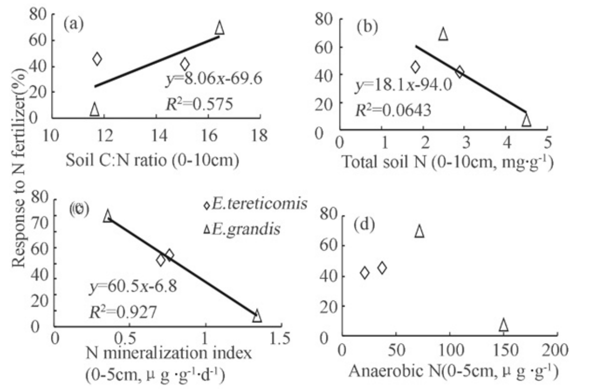

Fig. 4. Relationship between response to N fertilizer (a) soil C:N ratio, (b) total soil N, (c) N mineralization index in the surface (0-5 cm) soil, and (d) N released during an anaerobic incubation of the surface (0-5 cm) soil.

Modelled net N mineralization and response to fertiliser N

Modelling net soil N mineralization on an annual basis did not improve the diagnosis of fertilizer response, compared to the other soil indicators (Fig. 5). Results for the Surianelli site were consistent with low predicted annual net N mineralization. However, other 3 sites had similar predicted annual net N mineralization but widely variable response to added fertilizer N. The Vattavada site for example had a very low response to fertilizer N, but it had by far the largest demand and the high growth rate (Table 4). But *E. tereticornis* had a much lower demand (slower growing plantations), and were responsive to applied N fertilizer.

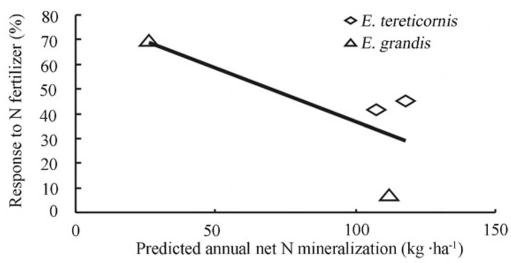

Fig. 5 Relationship between response to N fertilizer across the 4 sites and predicted annual net N mineralization.

Goncalves and Carlyle (1994) and O'Connell and Rance (1999) showed that it was feasible to model soil N mineralization through an understanding of the microbial responses to temperature and moisture, as well as knowledge of the basal rate of N mineralization, which is specific to a given soil. We extended their methodology to examine the utility of this technique for predicting a more general response to N fertilizer. The moisture and temperature response curves were of a similar shape in our study to those of O'Connell and Rance (1999). There was some variability around the moisture response, but the variability within sites was as much as the variability between sites, thus a common regression could be fitted to the data across the 4 sites. Interestingly, the upland soils with *E. grandis* were more responsive to temperature, having higher relative N mineralization. In

general, pools of anaerobic N in upland sites was high, however these sites experience low annual temperature variations. Thus higher response to temperature may be useful in explaining the lesser usefulness of high anaerobic N index for the inclusion in diagnostic tool and explaining the response to added N fertilizer in these sites.

Table 4. Comparison of predicted N supply and aboveground internal requirement (kg·ha^{-1}·a^{-1}) in low and high contents of N fertilizer

Eucalyptus species/sites	Predicted net N mineralization[a] (kg·ha^{-1}·a^{-1})	Annual N internal requirement[b] (kg·ha^{-1}·a^{-1})	
		Nil N	Sufficient N
E. tereticornis			
Kayampoovam	118.2	27.0	39.2
Punnala	107.2	25.9	36.3
E. grandis			
Surianelli	26.2	47.9	62.4
Vattavada	111.8	87.6	89.5

Notes: [a]Calculated for the 1999 calendar year in the 0−10 cm depth range; [b]Average of years 2, 3 and 4.

The predicted annual N mineralization rates for 26 kg·ha^{-1}·a^{-1} to 118 kg·ha^{-1}·a^{-1} (0-10 cm at depth) were generally similar to those found by O'Connell and Rance in south-western Australian soils (69–113 kg·ha^{-1}·a^{-1}, 0–20 cm at soil depth), but slightly lower than those found in other studies in tropical ecosystems. For example, Maithani et al. (1998) found annual rates of N mineralization for 138−162 kg·ha^{-1}·a^{-1} (0–10 cm) under sub-tropical rainforest in north-eastern India, and Smith et al. (1998) found rates (0–20 cm) for 195 kg·ha^{-1}·a^{-1} (*P. caribaea* plantation) to 328 kg·ha^{-1}·a^{-1} under native forest in Amazonia state, Brazil. The low N mineralization predicted at Surianelli (26 kg·ha^{-1}) is attributable to the low basal rate, which was probably a result of the site previously having been grassland.

In this study, the best indicator (ie. highest R^2) of response to fertilizer across the 4 sites was the net N released during an aerobic incubation (N mineralization Index, Fig. 4(c)). The Vattavada site had the highest productivity and thus the greatest demand for N. Predicted net annual above-ground N uptake by the stand was around 90 kg·ha^{-1}·a^{-1}, slightly less than the predicted 112 kg·ha^{-1}·a^{-1} mineralized from the soil. The predicted annual N mineralization at Surianelli was much lower than the observed net N uptake, thus explaining the strong response to applied N fertilizer. The two lowland sites of *E. tereticornis* had high levels of predicted net N mineralization and relatively low N requirement, but much of this N is predicted to be mineralized during the dry season (April to September), when soil moisture is likely to be limiting tree growth. We did not measure the seasonality of growth, however, such an analysis may be required to further resolve the supply and demand dynamics at these sites and assist in development of more suitable generic index for sites with relatively similar soil characteristics.

Conclusion

N released during an aerobic incubation was found to be the best index of a response to N fertilizer across the 4 Eucalyptus sites in this study. A modelled index based on the predicted annual N mineralization (using a function of soil moisture and temperature) was not as effective an index as the N released during an aerobic incubation. This may be due to the lack of synchrony between demand and supply as the trees have a marked seasonality to their growth and would have a high demand for N during their growing season. Further research is required to resolve whether matching N availability with demand on a sub-annual basis would improve this relationship.

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